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INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.

To: (Name and Address of Depositor or Attorney)

Elan Pharmaceuticals, Inc.
Attn: Nina Ashton
800 Gateway Boulevard
South San Francisco, CA 94080

Deposited on Behalf of: Elan Pharmaceuticals, Inc.

Identification Reference by Depositor:	**Patent Deposit Designation**
Murine Hybridoma Cell Line: RB44-10D5.19.21	PTA-5129
Murine Hybridoma Cell Line: RB96 3D6.32.2.4	PTA-5130

The deposits were accompanied by: __ a scientific description _ a proposed taxonomic description indicated above. The deposits were received April 8, 2003 by this International Depository Authority and have been accepted.

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Marie Harris

Marie Harris, Patent Specialist, ATCC Patent Depository

Date: June 2, 2003

cc: Joe Liebeschuetz
Ref: Docket or Case No.: 15270J-004720US

Exhibit 1

Exp Neurol 1992;118:117-125.
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Cognitive impairment in adults with Down's syndrome:

Similarities to early cognitive changes in Alzheimer's disease

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Article abstract—Postmortem studies of brains from adults with Down's syndrome (DS) reveal a dramatic age-dependent increase in the incidence of neuropathology associated with Alzheimer's disease (AD). By the age of 40 years, virtually all DS individuals have AD neuropathology. Documentation of cognitive correlates of this phenomenon has been difficult, partly because of the preexisting mental retardation in DS. In the current study, we compared a group of adults with DS, 22 to 51 years old, with a matched control group on various behavioral measures such as savings scores, which are known to be sensitive in detecting early dementia in AD patients. By using the short delayed savings score from the California Verbal Learning Test (a test of verbal memory), a subgroup of DS adults was identified as memory-impaired. This group demonstrated a decline in performance on various other cognitive tests with advancing age, whereas another group identified as having non–memory-impaired DS, and the non-DS controls, showed no evidence of decline with age. These results provide evidence for the presence of early dementia among adults with DS within an age range in which neuropathologic manifestations of AD are predicted to be developing.

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Down's syndrome (DS) is a genetic disorder involving an excess of chromosome 21 (trisomy 21 in approximately 96% of the cases), and those with DS constitute approximately 15% of the population with mental retardation.[1] Several investigators reported a discrepancy between the presence of neuropathologic hallmarks[2] of Alzheimer's disease (AD) and dementia[3,4] in DS. Based on results of postmortem studies, almost all individuals with DS have neurofibrillary tangles and neuritic plaques (changes associated with AD in nonretarded individuals) present in their brains by age 40 years. However, previous studies[3-10] identified dementia among only some DS subjects over 40 years old. In many of these studies, DS subjects defined as demented were compared with a group of DS subjects of similar age who may also have been exhibiting early changes of dementia not detected by the measures employed. Furthermore, the presence of preexisting mental retardation in most individuals with DS presents a challenge in the detection of dementia in this population.

The potential cognitive changes associated with dementia of the Alzheimer type (DAT) in DS are not well characterized nor have sensitive markers detecting early dementia been identified. Measures of memory function, particularly delayed recall or

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measures of the percent of memory retention over time (referred to as "savings scores") are sensitive and fairly specific in the detection of early DAT among the non-DS population.[11-15] Welsh et al[13,14] reported the savings score from the CERAD neuropsychological test battery to be the second best variable in discriminating between AD patients with *mild* DAT and nondemented age-matched controls, using various tests of memory and language (ie, the Boston Naming Test [BNT] and Category Fluency) and a test of constructional praxis. Delayed recall was the best detector of mild DAT in the study of Welsh et al. However, these investigators excluded from the statistical analysis data from subjects who recalled fewer than two items on short delay. Other investigators,[12] who did not employ this exclusionary criterion and obtained savings scores using the Logical Memory and Visual Reproduction subtests of the Wechsler Memory Scale-Revised (WMS-R), reported the savings score as the most sensitive and specific marker for early DAT. Intrusion errors or false positives that reflect other subtle aspects of memory function are reported to occur in the later stages of DAT.[13-15]

Children[16] or adults with DS,[17] when compared with control subjects of similar age and overall intelligence, show greater impairment in specific verbal tasks. Such impairment in nonmemory verbal performance may precede the onset of dementia in DS; therefore, nonmemory language tests such as Category Fluency and the BNT, which reveal impairments particularly in the *advanced* stages of DAT in AD patients,[14] may not be sensitive in detecting *early* dementia among DS adults.

The purpose of the present study was to determine whether cognitive impairments associated with early dementia in DS adults could be detected by measures that are sensitive in detecting dementia in AD patients. Since the DS subjects of the present study were within the age range in which the age-dependent increase of AD neuropathology in DS is known to be the greatest,[2] an age-dependent decline of memory function reflected by the savings score or delayed recall is predicted to exist among the DS but not the control subjects.

Methods. *Subjects.* Seventeen subjects with DS and seven age-matched, mentally retarded controls participated in the study. All subjects resided in two group homes in southern California. The mean age of the DS group, 31.1 ± 2.9 years (range, 22 to 51), was not significantly different from that of the seven controls, 28.9 ± 2.8 years (range, 22 to 46). The control subjects were also not significantly different from the DS group in mean Full Scale Intelligence Quotient (60.9 ± 2.8 for controls and 55.3 ± 1.6 for DS subjects), Performance IQ (controls, 64.9 ± 1.2; DS, 61.4 ± 1.5), or Verbal IQ (controls, 60.9 ± 2.9; DS, 55.5 ± 1.4) of the Wechsler Adult Intelligence Scale-Revised (WAIS-R).[18] All subjects had a complete physical evaluation, including neurologic and psychiatric examinations, and medical histories wer obtained. None of the subjects had active major medical disorders (such as heart disease, infections, lung disease, liver disease, kidn y disease, or diab tes) ther than the neurologic condition r sulting in moderate mental retardation. The diagnos s of th m ntally retarded controls included hydrocephalus with a normal-pressure shunt, translocation of chromosomes 5/X r chr m somes 19/20, microcephaly, c rebral palsy, and unknown etiologies of mental retardation. All were ambulatory and active in daytime workshops or employment programs. Routine laboratory blood tests on each subject included CBC, BUN, creatinine, glucose, platelets, and chemistry profile and were within normal limits in all subjects. Since DS is associated with a high incidence of hypothyroidism, blood thyroxine and T_3RU levels were obtained. Although four DS subjects were taking thyroxine, all subjects showed thyroxine and T_3RU levels within the normal range. One DS subject and four controls were taking anticonvulsant medication. However, each of these had had their most recent seizure more than 1 month prior to the time of study, and most of them had had their last seizure several years prior to the study. Four of the seven control subjects were receiving a low dose of psychotropic m dication such as 1 mg of haloperidol (orally) daily or a tricyclic antidepressant, and three were on medroxyprogesterone or primidone. Five DS subjects and three controls were taking daily vitamins, typically multivitamins. Subjects taking these various medications performed within two standard deviations of their respective group means on all neuropsychological test variables. All subj cts were nonsmokers and on a similar diet, provided in th group home. None was obese.

Neuropsychological testing. All subjects were administered the following battery of neuropsychological tests: WAIS-R,[18] Logical Memory and Visual Reproduction subtests of the WMS-R,[19] California Verbal Learning T st children's version (CVLT-C),[20] BNT,[21] Peabody Picture Vocabulary Test-Revised (PPVT-R),[22] Memory for S ntences (Memsent) and Memory for Objects from the Stanford-Binet Intelligence Scale, fourth edition,[23] Letter (Controlled Oral Word Association Test) and Category (Animals) Fluency tests,[24] Beery Developmental T st of Visual-Motor Integration,[25] Opposites subtest of the McCarthy Scales of Children's Abilities,[26] and Finger Tapping for the dominant (Tap Dom) and nondominant (Tap NonDom) hands from the Halstead-Reitan Battery.[27] The difference between the dominant and nondominant hand on Finger Tapping (DifferTap) was also obtained. The number of intrusion errors during generation of words starting with the letters "F," "A," or "S" on the Letter Fluency test was recorded and referred to as "FAS intrusions." The data on some subtests from one of th seven controls and one DS subject were missing or excluded due to the subject's unwillingness to respond.

The Logical Memory subtest from the WMS-R assessed immediate (I) and delayed (30-minute delay; II) recall of two paragraphs, A and B (Logical Memory IA and IB and Logical Memory IIA and IIB). A savings scor for Logical Memory (expressed as percentage) was obtained with the following formula: Logical M mory IIA + IIB/Logical Memory IA + IB multiplied by 100. The Visual Reproducti n subtest assess d immediate (VRIAD) and delayed (30-minute delay; VRIIAD) recall of four designs (designs A through D). A savings score for Visual Reproduction (expressed as percentage) was obtained by calculating the f ll wing: VRIIAD/VRIAD × 100.

The children's v rsi n f the CVLT was ch sen to av id the floor ff ct that might be obtained with the adult versi n. Sinc this test is not widely used, we provide a description. The CVLT-C is a verbal list-learning

task that provides measures of several aspects of learning and memory. On each of five trials, 15 words (list A) were presented orally at the rate of one word per second, and immediate free recall of the words was elicited. A second list (list B) was presented for one trial immediately after trial 5. Following the list B trial, the subject was asked to again recall the items of list A (short delayed free recall) and then to recall the words from list A with semantic cues provided (short delayed cued recall). After a 20-minute interval during which unrelated nonverbal tests were administered, the subjects were given free recall and category-cued recall trials (long delayed free recall and long delayed cued recall, respectively) and a recognition test of list A. The yes/no recognition test consisted of 30 distractor items intermixed with the 15 items of list A. The number of false-positive responses on the recognition test and the number of intrusions over all test trials were recorded. Savings scores for short and long delayed recall (expressed as percentages) were obtained by dividing short delayed free or long delayed free recall, respectively, by trial 5 recall multiplied by 100.

Statistical analysis. A stepwise logistic regression analysis comparing the DS and control subjects was performed with measures known to be sensitive to cognitive impairment in the early stages of Alzheimer's disease (hereafter referred to as "Alzheimer-related variables"). Stepwise logistic regression serves the same purpose as stepwise discriminant analysis in that it uses explanatory variables to determine to which of two groups (DS or control) each individual in the population belongs. The major difference is that, unlike discriminant analysis, logistic regression does not assume that the explanatory variables are normally distributed within each group. Since this assumption was obviously violated for several of the variables we were considering, we chose logistic regression as the better technique to use. The variables included in this analysis were Logical Memory IIA and IIB; VRIAD, VRIIAD, and the Visual Reproduction savings score from the WMS-R; false positives; intrusions on short cued recall and long cued recall; short and long delayed savings scores and trials 1-5 recall from the CVLT-C; FAS intrusions from the Letter Fluency test; and the score from the BNT. The Logical Memory savings score was not included in this analysis since several subjects received a score of zero on the Logical Memory immediate recall condition. Short and long free recalls of the CVLT-C were not included among the Alzheimer-related variables due to a floor effect of these variables among the DS subjects (raw mean scores of the DS subjects were 34 ± 1.0 and 4.2 ± 1.2; the raw mean scores of the control subjects were 6.2 ± 0.8 and 5.0 ± 1.4).

The stepwise logistic regression model was designed to determine which of the above variables best discriminates between DS and control subjects. The procedure considered all the Alzheimer-related variables and entered the best discriminating variable first in the model. Subsequently, the model entered the second variable with the greatest additional discriminating power (while considering all the Alzheimer-related variables) in the presence of the first variable. Due to an insufficient number of subjects, the third best discriminating variable could not be entered in the model. Since this analysis could not provide reliable group differences beyond the two best discriminating variables, separate t tests were performed to compare the performance of the DS and control groups on each of the Alzheimer-related variables. Statistical analysis on all psychometric variables in the present study was performed on raw scores. Since the DS subjects were predicted to show a greater impairment of performance compared with controls, the level of significance for a one-tailed test is reported.

Results. *Comparisons of the DS and control groups on Alzheimer-related variables.* Despite equivalent levels of general intellectual ability, DS subjects were significantly more impaired than the mentally retarded control subjects on the following Alzheimer-related variables: short delayed savings score ($p < 0.001$), long delayed savings score ($p < 0.01$), false positives ($p < 0.025$), BNT ($p < 0.025$), VRIAD ($p < 0.05$), and intrusion short and long cued ($p < 0.05$ for both measures). Using the Bonferroni correction (a criterion of $p < 0.001$ to be significant), the short delayed savings score was the only Alzheimer-related variable to show a significantly greater impairment in the DS group. With the Bonferroni correction, other variables showed trends for greater impairment in DS compared with controls. Short and long free recall failed to show significant differences between the DS and control groups due to floor effects. The DS group also showed significantly greater impairment than did controls on the following psychometric variables not included among the Alzheimer-related variables: DifferTap, PPVT, WAIS-R similarities, and Memsent (ranging from $p < 0.05$ to 0.001). (The mean raw diagnostic group scores and the results of the t test comparisons without Bonferroni correction between the DS and control groups on the Alzheimer-related variables and on other psychometric variables are in table 1, filed with the National Auxiliary Publications Service [NAPS]; see Note at end of article.)

A stepwise logistic regression analysis on Alzheimer-related variables revealed that the short delayed savings score from the CVLT-C was the variable that best discriminated between DS subjects and controls ($p < 0.005$, $\chi^2 = 9.30$), in that it was the only variable to show a group difference sufficient to enter the model. Among all psychometric variables, the short delayed savings score was the only variable to be significantly ($r = -0.544$, $p < 0.02$) inversely correlated with age in the DS group but not in the controls (see table 2, filed with NAPS). The control group showed either no correlation or significant positive correlations of Alzheimer-related variables with age (Logical Memory IIA, $r = 0.847$, $p < 0.05$, and Logical Memory IIB, $r = 0.822$, $p < 0.025$). Furthermore, unlike the DS group, the control group showed a significant positive correlation (ranging from an $r = 0.691$, $p < 0.05$, to $r = 0.862$, $p < 0.01$) of Logical Memory IA and IB subtests and WAIS-R Comprehension, Information, and Vocabulary subtests, and a trend for a positive correlation of the Picture Completion subtest ($r = 0.640$) with age (see table 2, filed with NAPS).

Segregation of DS subjects into memory-impaired and memory-nonimpaired groups. Performance on the short delayed savings score was chosen as the cognitive measure to theoretically classify the DS subjects into a demented ("memory-im-

paired") or nondemented ("memory-nonimpaired") category since this variable was found to be (1) the only age-dependent variable in the DS subjects among more than 25 dependent variables, (2) not age-dependent in controls, (3) the variable that best discriminated DS from controls based on stepwise logistic regression analysis, and (4) a sensitive cognitive marker for *early* dementia in AD patients.

The memory-impaired DS subjects were defined as those with a short delayed savings score of zero (no retention after a short delay), whereas the memory-nonimpaired DS subjects had short delayed savings scores of greater than zero (ranging from 67% to 167%). The range of this savings score among the controls was 27% to 100%. Since one DS subject failed to recall any items on trial 5, the data for this subject were excluded in subsequent analyses involving the two DS subgroups. A fairly even distribution of subjects was observed among the three diagnostic groups: controls (N = 7), memory-impaired (N = 7) and memory-nonimpaired DS (N = 8) groups. The term "memory-nonimpaired" is relative in this context in that the memory-nonimpaired DS and the control groups were not significantly different in their mean short delayed savings scores (77.4 ± 7.5 for the memory-nonimpaired DS group and 110.36 ± 14.6 for the control group).

The two DS subgroups did not differ in mean Full Scale IQ (54.3 ± 1.9 for the memory-impaired and 57.1 ± 2.9 for the nonimpaired group), Performance IQ (61.7 ± 1.8 for the memory-impaired and 62.3 ± 2.9 for the nonimpaired), or Verbal IQ (54.4 ± 1.7 for the memory-impaired and 57.1 ± 2.4 for the nonimpaired). However, the memory-impaired DS subjects were significantly ($p < 0.01$, $t = 3.016$, $df = 13$) older than the memory-nonimpaired DS subjects (mean ages, 38.6 ± 3.8 years for the memory-impaired and 27.9 ± 1.4 years for the nonimpaired). Although the control group had a mean age (28.9 ± 2.8 years) similar to that of the memory-nonimpaired group, it did not differ significantly from either DS subgroup.

Comparisons between memory-impaired and memory-nonimpaired DS groups and controls on various measures of cognitive performance. Comparisons (using ANOVA) among the three diagnostic groups on various psychometric measures revealed main effects among the three groups for most of those variables in which significant differences existed between the controls and the entire DS group (see table 1, filed with NAPS). The Alzheimer-related variables showing diagnostic group differences included the short delayed savings score ($p < 0.001$), long delayed savings score ($p < 0.01$), false positives ($p < 0.025$), and VRIAD ($p < 0.05$). Other Alzheimer-related variables, such as intrusions (FAS, and short and long cued) failed to show significant diagnostic group differences. In addition, a significant group effect was observed for three other measures of memory function, short free recall ($p < 0.001$), long free recall ($p < 0.05$), and Memsent ($p < 0.025$), and for a psychomotor measure, DifferTap ($p < 0.025$). BNT, PPVT, and the WAIS-R similarities subtest showed trends for diagnostic group effects ($p < 0.075$, $F = 2.94$).

Post hoc t tests were performed on those variables showing significant diagnostic group effects by ANOVA. Memory-nonimpaired DS subjects failed to differ significantly from the control subjects on almost all the variables. In contrast, the memory-impaired DS group was significantly worse than control subjects on the following variables: short and long delayed savings score ($p < 0.001$), short ($p < 0.001$) and long ($p < 0.05$) free recall, false positives ($p < 0.05$), VRIAD ($p < 0.05$), Memsent ($p < 0.01$), and DifferTap ($p < 0.01$) (table 1, filed with NAPS). The magnitude of impairment exhibited by the memory-impaired DS group tended to be greater than that of the memory-nonimpaired DS subjects on several variables. However, only the short ($p < 0.001$) and long ($p < 0.025$) delayed savings scores and short free recall ($p < 0.001$) showed significant differences between the two DS subgroups.

Correlation of neuropsychometric measures with age in memory-impaired DS, memory-nonimpaired DS, and controls. Since the DS group as a whole failed to show significant correlations with age on any variable while the controls showed positive correlations, it was hypothesized that the memory-impaired subgroup included in the larger DS cohort was masking age effects that might exist in the memory-nonimpaired DS. If this were the case, nonimpaired DS would be expected to show correlations similar to those observed in the controls (ie, improvement of WAIS-R verbal subtests with age) while memory-impaired DS would be expected to show no correlation or a decline in performance with age. Pearson product-moment correlations of several variables with age for the three diagnostic groups are shown in figures 1 through 3. (Pearson product-moment correlations for Alzheimer-related variables, WAIS-R subtests, and other psychometric variables are shown for all diagnostic groups in table 2, filed with NAPS.)

Segregation of the DS groups into memory-impaired and nonimpaired subgroups revealed that the nonimpaired DS subgroup showed significant positive correlations or trends for positive correlations between age and various WAIS-R verbal subtests (ranging from $r = 0.645$ to 0.735, $p < 0.05$ to 0.025) and tests of memory (Logical Memory IB, $r = 0.640$, $p < 0.05$), similar to those of the controls (ranging from $r = 0.691$ to 0.862, $p < 0.05$ to 0.01 for WAIS-R verbal subtests, and ranging from $r = 0.780$ to 0.861, $p < 0.05$ to 0.01 for all four Logical Memory subtests). In contrast, the memory-impaired DS group failed to show improvement in cognitive function with age on any of the variables. Instead, the memory-impaired DS subgroup showed a significant decline, or trend for a decline (ranging from $r = -0.668$ to -0.835, $p < 0.05$ to 0.01), in cognitive performance with advancing age on a number of measures. These measures included several Alzheimer-related variables, other m asures of memory function (Logical Memory subtests, trial 1-5 recall, list B recall), and nonmemory variables including the

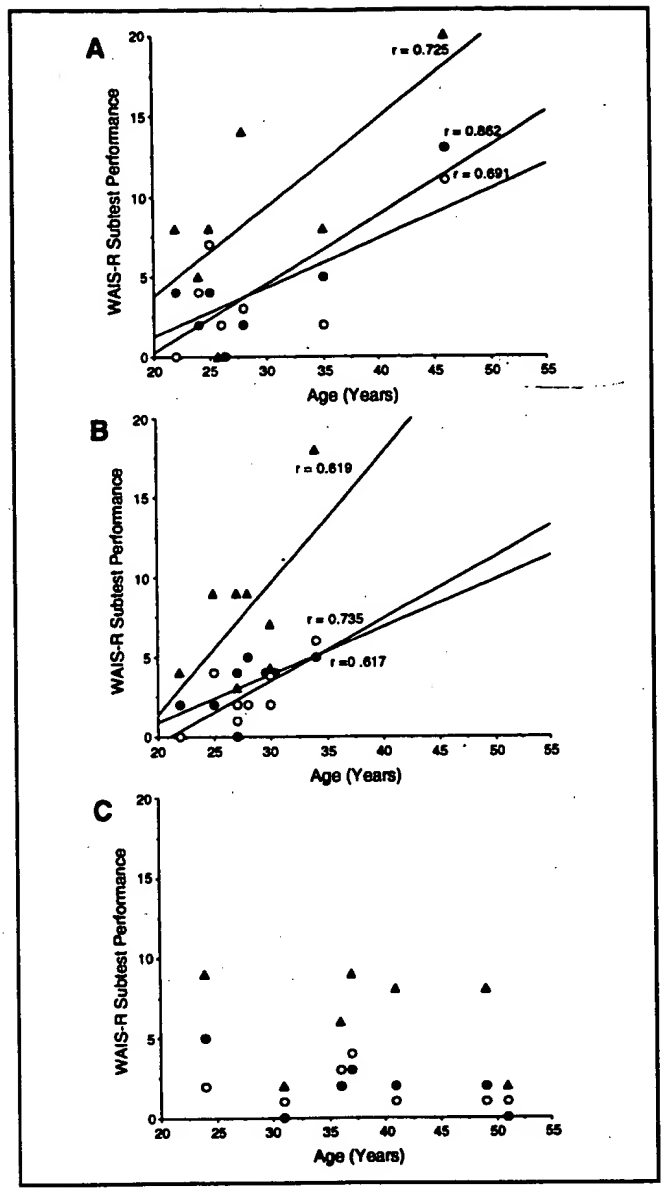

Figure 1. Correlations of age with performance on the WAIS-R subtests; Information (open circle), Vocabulary (triangles), and Comprehension (closed circles) for each diagnostic group: (A) controls, (B) memory-nonimpaired Down's, and (C) memory-impaired Down's.

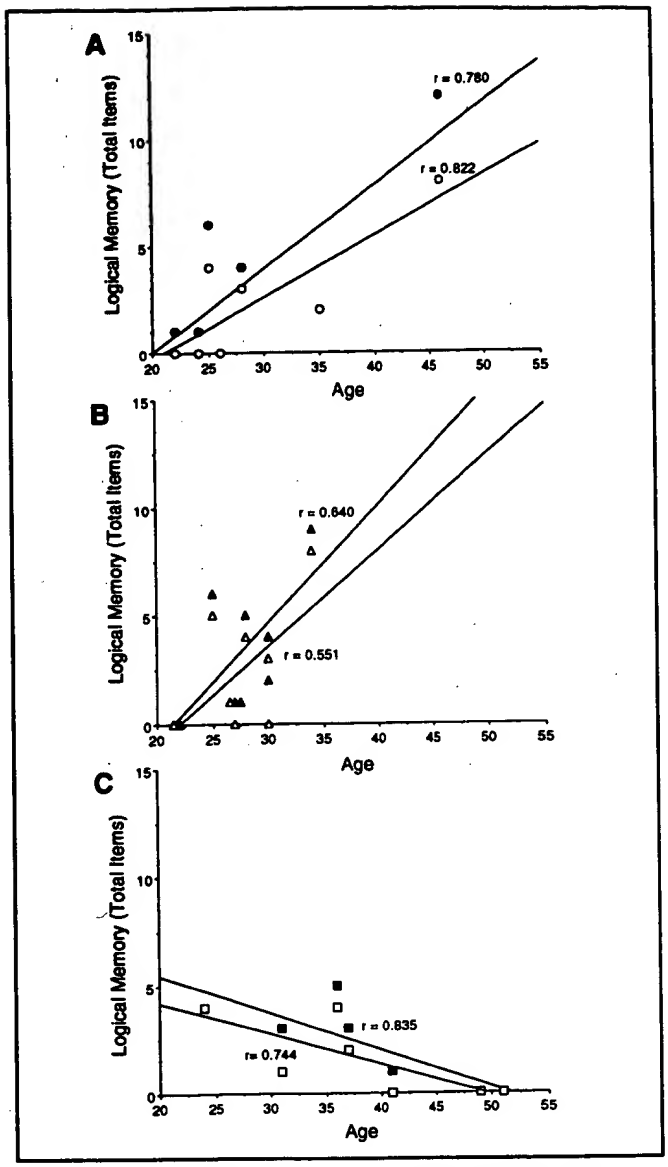

Figure 2. Correlations of age with immediate Logical Memory recall (closed circles) and delayed Logical Memory recall (open circles) of part B of the Wechsler Memory Scale-Revised for each diagnostic group: (A) controls, (B) memory-nonimpaired Down's, and (C) memory-impaired Down's.

WAIS-R Block Design subtest and Finger Tapping (for Tap NonDom and Tap Dom, see figure 3; see also table 2, filed with NAPS).

Discussion. In the present study, the short delayed savings score (obtained from the CVLT-C), a sensitive cognitive marker for early DAT in AD patients,[12-15] (1) declined with advancing age in the DS group but not in the controls, and (2) discriminated DS from controls based on stepwise logistic regression analysis. Other psychometric measures, which reveal impairment in the *later* stages of DAT,[14] such as false positives, intrusion errors, and performance on the BNT, failed to show sufficient impairment in DS compared with controls to enter into the stepwise regression analysis model. When separate comparisons were made for each cognitive variable, several variables, such as intrusion errors, revealed greater impairment in the DS group than in the controls but failed to decline with advancing age among the DS subjects. Intrusion errors occur in a variety of neurologic conditions[28] in addition to AD, and may reflect a more static memory deficit that antedates the onset of more progressive changes of dementia (as seen in older DS adults), such as a decline in the savings score. A decline in short delayed free recall performance with age was not observed among the DS group, perhaps due to a floor effect on this memory function.

There are a number of caveats in the present

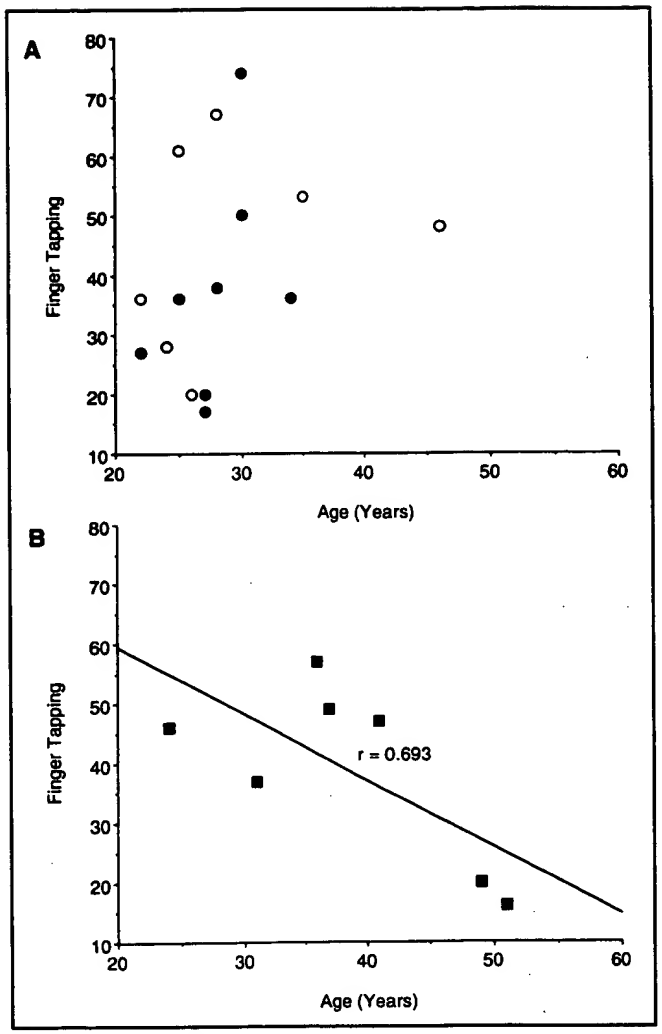

Figure 3. Correlations with age of Finger Tapping (the sum of finger tapping of the dominant and nondominant hands) for each diagnostic group: control (open circles) and memory-nonimpaired Down's (closed circles) subjects in (A) and memory-impaired Down's subjects in (B).

study. First, since DS subjects with severe cognitive impairment could not participate in the neuropsychological testing, the results of the present study apply only to a subpopulation of DS. Second, some of the results of the present study are reported without Bonferroni correction. Third, the study lacked a longitudinal design, which would be necessary to demonstrate a progressive decline of cognitive function among the memory-impaired DS subjects. However, a sensitive marker for DAT[12-15] was employed and revealed an age-dependent decline in performance among DS subjects but not controls, who were within the age range in which the rate of appearance of AD neuropathology in DS is predicted to be greatest.[2]

Impairments in several aspects of verbal function, such as performance on the PPVT, are present in DS subjects compared with controls of similar overall intelligence and age,[16,17] and were also observed in the present study. Impairment of verbal skills appears to precede the development of dementia in DS, since it occurs during childhood years.[16] Consistent with these observations, the DS adults of the present study failed to show an age-dependent decline in performance on the PPVT. Other measures of verbal abilities also failed to discriminate DS from controls or failed to show an age-dependent decline among the DS subjects. Furthermore, the memory-impaired DS group failed to show greater impairment in nonmemory verbal tests than did the controls or the memory-nonimpaired DS group. Therefore, nonmemory verbal tests did not appear to be sensitive in detecting early dementia among DS adults in the present study. Non-DS AD patients generally show impairment on nonmemory verbal tests to a greater extent in advanced rather than early stages of DAT.[14]

If the short delayed savings score is a sensitive marker of early dementia in DS, then DS subjects identified as memory-impaired based on their short delayed savings score performance might also exhibit a decline in performance on other cognitive functions with age. Indeed, a decline in performance on a number of psychometric variables with increasing age that failed to exist for the entire DS group was unmasked among the memory-impaired DS subjects but not among the nonimpaired DS subjects or controls. Similarities in verbal and nonverbal levels of intelligence of these two DS subgroups suggest that any differences between the DS subgroups cannot be attributed simply to a lower baseline level of intelligence among the memory-impaired group. Furthermore, the short delayed savings score, which was used to identify the memory-impaired DS subjects, did not correlate with WAIS-R verbal ($r = 0.238$) or performance ($r = 0.102$) IQ scores but instead correlated with age, which is consistent with the age-dependent development of dementia.

Because the memory-impaired DS group was significantly older than the memory-nonimpaired group, differences between these two DS subgroups might be attributed to age effects rather than to the development of dementia. However, since the short delayed savings score is sensitive in detecting early DAT in AD patients[2,15] and it declined with advancing age among DS adults, then it appears that this savings score is reflecting memory deficits associated with dementia in DS adults who are known to be developing AD neuropathology, rather than reflecting aging. In the present study, the savings score does not decline with advancing age among healthy elderly subjects[29] nor among the non-DS developmentally disabled subjects, in contrast to DS subjects. Furthermore, an age-dependent decline of Logical Memory performanc , associated with DAT in the non-DS population, was observed among the memory-impaired nonelderly DS but not among the controls in the present study nor among the nongeriatric (ages 20 to 59) non-DS adults of previous studies.[30] Thus, it appears that differences in cognitive deficits among memory-impaired and nonimpaired DS cannot be sufficiently explained by age effects alone.

An acceleration of neurodegenerative pathology associated with aging might explain the age-dependent decline of performance on nonverbal subtests of the WAIS-R or Finger Tapping on the Halstead-Reitan within the "impaired" subgroup of DS, since these tests are reported to decline with age in the healthy population.[31] Based on numerous reports of the premature onset of a variety of age-dependent phenomena and diseases,[2,32-34] acceleration of aging in DS is believed to exist.

The results of the present study are consistent with the presence of early dementia among DS adults, some of whom are younger than 40 years, and who are within the age range in which the rate of appearance of AD neuropathology is known to be greatest.[2] However, a longitudinal reexamination of the subjects in the present study is required to confirm this hypothesis. Utilization of measures of subtle aspects of memory function, such as the short delayed savings score, should be considered for future studies that attempt to categorize adults with DS into demented and nondemented groups, particularly studies examining potential biochemical correlates of dementia in this population.

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Down Syndrome and Alzheimer Disease, pages 123-142

DOWN SYNDROME AND ALZHEIMER DISEASE

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Summary

The brains of individuals with Down's syndrome in their 40's and 50's begin to develop changes that are otherwise seen only in patients with Alzheimer disease. Neurons develop neurofibrillary tangles, flame-shaped alterations composed mainly of condensed cytoskeletal proteins. Another protein, β/A4 amyloid, is deposited in large amounts in the form of senile plaques and, around blood vessels, amyloid angiopathy. With increasing age, Down syndrome individuals accumulate more and more of these changes.

Different parts of the brain are affected to varying degrees by these two alterations. Surprisingly, the pattern of accumulation of neurofibrillary tangles and senile plaques is characteristic, and follows a predictable pattern. We have characterized this pattern in the hippocampal formation in a group of Down individuals, ages 13-71. Certain specific neurons such as those in layer II of entorhinal cortex and the CA1/subiculum field of the hippocampus are exquisitely vulnerable to tangle formation, and are the first neurons to be affected. Perhaps 20-30 years pass as the disease process evolves from mild to severe pathological changes.

One hypothesis for why Down individuals would be predisposed to developing Alzheimer pathology is the observation that the gene that encodes the precursor of the amyloid protein is located on chromosome 21. An extra copy of this gene, such as occurs in Down syndrome, may lead to "overproduction" of amyloid, and ultimately to its accumulation as senile plaques. Experiments to test this hypothesis are now underway.

Introduction

Down syndrome, the clinical manifestation of trisomy of chromosome 21, is a complex of physical signs that include a spectrum of mental retardation, congenital heart disease, dermatoglyphic changes and an early predisposition to developing the neuropathological and clinical features of Alzherimer disease. It is a common illness, occurring in approximately 1/1000 live births. As Mann (1988) has pointed out, the life expectancy of an infant with Down syndrome was only 9 years in 1929, whereas now with more aggressive treatment of infectious disease as many as 70% of individuals with Down syndrome can expect to live beyond age 50. A individual with Down syndrome live longer, it has been recognized that with increasing age essentially all middle aged Down individuals develop neurofibrillary tangles and senile plaques, the neuropathological hallmarks of Alzheimer disease. This review will summarize what is known about the relationship of Alzheimer disease and Down syndrome.

Clinico-pathological correlations

Down syndrome and Alzheimer disease are closely interrelated. After age 35, essentially 100% of patients with Down syndrome develop the neuropathological changes of Alzheimer disease (Burger and Vogel, 1973; see Kemper, 1988 for review). By contrast, in the general population neurofibrillary tangles and senile plaques are extremely rare until the mid-50's, and even then occur only in small numbers (Arriagada et al., 1991). Wisniewski and colleagues (1985) examined 100 brains of patients with Down syndrome who were 1 to 74 years of age. Neurofibrillary tangles and senile plaques were found in 49 of 49 patients above the age of 30, and in 7 of 49 below that age. Ropper and Williams (1980) found plaques and tangles in all 20 cases of Down syndrome, examined at ages 30-69 years. It is also well documented that middle-aged individuals with Down syndrome commonly develop cognitive impairment in a pattern similar to that of Alzheimer disease. For example, Wisniewski et al. (1985b) provided a clinicopathological description of seven patients with Down syndrome over the age of 40 with documented progressive cognitive impairment extending from 2.5 to 9 years before death. At autopsy, each of these patients was shown to have developed severe accumulation of neurofibrillary tangles and senile plaques. Lai and Williams (1989) prospectively evaluated 96 patients with Down syndrome and found an increasing prevalence of dementia with age. Functional decline was seen in 55% of patients between 50 and 59, and 75% of patients over 60. Brains from all 12 autopsied cases (average age 62) showed large numbers of plaques and tangles, in the same locations as persons with Alzheimer disease. A study of 50 adult Down patients with mild mental retardation confirmed these figures. The prevalence of dementia increased from 0 in the group age 20-29, to 33% in the age group 30-39 to 55% in the age group 40-52, and all demented patients had signs of brain atrophy on CT (Franceschi et al., 1990). In our own study (Hyman and Mann, 1991) of 20 Down individuals ages 13-71, we found mild to moderate Alzheimer neuropathological changes in individuals ages 31-50, and marked changes in all individuals over age 50. Cognitive decline had been noted in all patients over the age of 58 (Table 1). Thus, mild or moderate neuropathological changes did not manifest as overt loss of cognitive ability, implying a presymptomatic stage of Alzheimer-type dementia that lasts 20-30 years.

Table 1. Characteristics of Down syndrome individuals[1]

	Age	Clinical Status[2]	Neuropathology[3]
DS-1	13	ND	None
DS-2	31	ND	Mild
DS-3	37	ND	Mild
DS-4	38	ND	Mild
DS-5	40	ND	Mild
DS-6	41	UNK	Mild
DS-7	42	ND	Mild
DS-8	47	ND	Moderate
DS-9	49	ND	Mild
DS-10	50	PrD	Moderate
DS-11	52	UNK	Marked
DS-12	53	ND	Marked
DS-13	58	D	Marked
DS-14	59	D	Marked
DS-15	59	D	Marked
DS-16	60	D	Marked
DS-17	60	D	Marked
DS-18	64	D	Marked
DS-19	65	D	Marked
DS-20	71	D	Marked

[1]Adapted from Hyman and Mann, 1991.
[2]D, demented; ND, not demented; PrD, probable dementia; UNK, unknown.
[3]As judged by neurofibrillary tangle accumulation in the hippocampal formation.

Patterns of neurofibrillary tangle and senile plaque pathology in Alzheimer disease and Down syndrome

Neurofibrillary tangles. It has been known for many years that neurofibrillary tangles and senile plaques preferentially accumulate in some areas of the cortex, and other areas tend to be spared (Hirano and Zimmerman, 1962; Hyman et al., 1984; Saper, Wainer and German, 1987; Rogers and Morrison, 1985; Arnold et al., 1991). Recently, information gained from the study of neural connections in experimental animals has been applied to the topography of Alzheimer changes, allowing a re-analysis of the patterns of pathology (Hyman et al., 1984; Van Hoesen and Damasio, 1987 for review). This has led to the conclusion that specific sets of neurons, particularly projection neurons in defined cytoarchitectural fields in the hippocampal formation and association cortices, consistently develop neurofibrillary tangles (Hyman et al., 1984; Rogers and Morrison, 1985; Pearson et al., 1985; Arnold et al., 1991). Other neurons (for example, those in primary sensory or motor cortices) are resistent to neurofibrillary tangle degeneration.

The population of vulnerable neurons is remarkably consistent from case to case. For example, neurons that seem to be primarily responsible for providing afferent and efferent information to the hippocampal formation are severely affected by neurofibrillary changes (Hyman et al., 1984;1986, 1990) (Figure 1). These include layers II and IV of entorhinal cortex, and the CA1/subicular zone of the hippocampus. Other fields, sometimes immediately adjacent, are relatively spared. The cytoarchitectural fields of the hippocampal formation are involved in a consistent hierarchical fashion. Our recent semiquantitative studies showed that the following order of involvement was obeyed in each of 22 cases of Alzheimer disease assessed : Entorhinal cortex (layer II) > CA1, Subiculum , EC layer IV > CA3, CA4 > dentate gyrus, presubiculum (Hyman et al., 1992). Is this also the case in Down's syndrome? Elderly Down syndrome individuals who have developed Alzheimer pathology clearly accumulate tangles in the same hierarchy and in the sam set of vulnerable neurons (Ball and Nuttall, 1980; Mann et al., 1986; Hyman and Mann 1991) The pattern of tangles in young Down patients has also been evaluated from this perspective, and thes same areas of entorhinal cortex and hippocampus

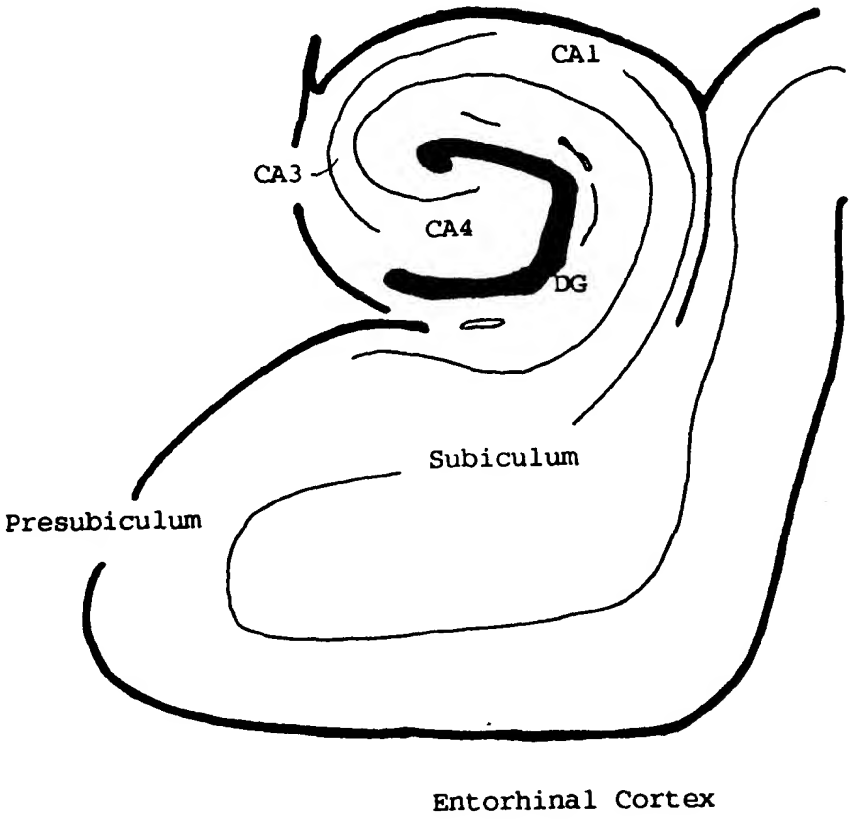

Figure 1. Anatomy of the human hippocampal formation. The majority of cortical input to the hippocampus is received by neurons in the entorhinal cortex. From here, the perforant pathway projects to the dentate gyrus (DG). A series of sequential intrinsic projections initiates from DG to CA3 (mossy fibers), then CA3 to CA1 (Schaeffer collaterals), then CA1 to subiculum (ammonic-subicular fibers). Primary output to cortex and limbic structures arises from areas CA1 and subiculum (see Hyman et al., 1990 for review).

appear to be vulnerable (Mann and Esiri, 1988; Hyman and Mann, 1991).

Significant advances have been made in the last five years in the biochemistry and molecular biology of neurofibrillary tangles. The primary identified component of tangles appears to be th microtubule associated protein, tau (Grundke-Iqbal et al., 1986; Wood et al., 1986 Kosik et al., 1986;1989; Nukina and Ihara, 1986 ; Wischik et al., 1988; Kondo et al., 1988; See Selkoe 1989 for review). Tau is a normal cytoskeletal protein that in the mature brain is located only in an axonal distribution. Its normal function appears to be to play a role in maintenance of neurite polarity (Caceres and Kosik,1990). In Alzheimer disease and in Down syndrome (Joachim, 1987; Flament et al., 1990), tau immunoreactivity appears in the somatodendritic compartment in association with neurofibrillary tangles. Neurons that contain neurofibrillary tangles remain capable of synthesizing tau, and <u>in situ</u> hybridization studies have shown that the mRNA for tau is present in neuronal soma and proximal dendrites (Kosik et al., 1989; Hyman et al., 1992)).

<u>Senile plaques.</u> A similar topographic hierarchical distribution can be mapp d for senile plaques (Hyman et al., 1986;1992). In the Alzheimer hippocampus a discrete hierarchical distribution is found with CA1, Subiculum > dentate gyrus (molecular layer), entorhinal cortex layer III > CA4, CA3, presubiculum . Moreover, our results suggests that frequently senil plaques and A4 amyloid deposition are found in th terminal zones of degenerating neurons (Hyman et al., 1986; 1990). Immunocytochemical evidence from Dr. Masters' laboratory (Davies et al., 1988) suggests that A4 amyloid protein is deposited in the brain of individuals in the normal population over the age of 45, and increases in amount over the next one to two decades By age 80, over 80% of the population had A4 deposition. Thus it appears that, in the normal population, A4

deposition is one of the earliest manifestations of aging or of Alzheimer disease. Of importance to understanding the development of Alzheimer changes in Down syndrome, the same group has reported a similar pattern occurring in Down syndrome, but the age when A4 deposition begins is three decades earlier, in the teenage years (Masters and Beyreuther, 1988; Mann et al., 1990).

One major biochemical component of senile plaques is the β-A4 amyloid protein, first isolated by Glenner and Wong, 1984, and Masters et al. 1985. This 40 or 42 amino acid hydrophobic peptide is derived from amyloid precursor proteins (APP),. a family of alternatively spliced proteins of 563,695,751,or 770 amino acids See Tanzi, et al., 1989 for review). The 563, 751,and 770 forms all contain an insert of amino acids homologous to the Kunitz class of protease inhibitors, and so may have a different biological function than that of the 695 form (Tanzi et al., 1988). In situ hybridization studies using autoradiography (Bahmanyar et al., 1987; Lewis et al. 1987) have shown that neurons contain mRNA for APP, but that there is no clear relationship between the distribution of neurons that are at risk for tangle formation and those that have APP message. We have recently shown by both in situ hybridization and immunocytochemistry that both KPI containing and the 695 forms are present in neurons, and both contribute to the dystrophic neurites present surrounding some senile plaques (Hyman et al., 1992; Tanzi and Hyman, 1992). Neurons that contain tangles appear to also show APP mRNA (all 4 transcripts) and apparently continue to synthesize APP.

Initial Alzheimer changes in Down syndrome. Neuropathologic examination of young individuals with Down syndrome who have died before the fifth decade show a few tangles and plaques. According to the idea of hierarchical vulnerability, we predicted that these first neurofibrillary

tangles would occur in neurons in layer II of entorhinal cortex and the CA1/subicular field.

Preliminary evidence suggests that it is exactly this population of neurons that tends to develop neurofibrillary tangles at an early age in Down syndrome (Mann and Esiri,1988; Hyman and Mann, 1991). Plaques tend to accumulate in the CA1 and subicular fields, and in the molecular layer of the dentate gyrus (Hyman and Mann, unpublished).

One clue to the early changes that a cell undergoes before neurofibrillary tangle degeneration is provided by an immunocytochemical tool, Alz-50. This is a monoclonal antibody raised against an Alzheimer brain homogenate and selected for the ability to recognize Alzheimer, but not control, brains (Wolozin et al., 1986; Hyman, 1988). The antibody recognizes both A68, a 68kD soluble protein (Wolozin et al., 1986), which is likely an epitope of the tau molecule (Lee et al., 1991). The antigen is also present in Down brains from the second decade onward (Wolozin, 1988; Mattiace, 1989). Our studies in Alzheimer disease (Hyman et al, 1988) and in Down syndrome (Hyman and Mann, 1991) support the idea that neurons at risk to develop neurofibrillary tangles become immunoreactive for Alz-50.

Alz-50 and anti-tau immunocytochemistry are useful for visualizing neurofibrillary tangles, dystrophic neurites, and some neurons that ar believed to be "pre-neurofibrillary tangles". Combining the power of the hierarchical vulnerability scheme we have developed in our studies of Alzheimer disease in which we can rank order cytoarchitectural fields in terms of involvement, and the predictive power of progression of Alzheimer pathology in the Down individuals, the hypothesis that Alz-50 and anti-tau recognize neurons that are destined to develop neurofibrillary pathology can be tested. As noted above, our initial studies suggest that Alz-50 does recognize "at-risk" neurons in Down syndrome (Hyman and Mann, 1991). These "at risk" neurons

show a diffuse stain over the cytoplasm which extends into the dendritic arborizations and the axon, giving an almost Golgi-like appearance (see, for example, Hyman et al., 1988). The immunohistochemical staining is not condensed as on sees with a neurofibrillary tangles, and the "at risk". Neurons can be shown not to contain a tangle by subsequent histological staining. Nonetheless, this is an abnormal staining pattern insofar as neither Alz-50 nor anti-tau immunocytochemistry reveals any staining in the somatodendritic compartment under normal circumstances. Alz-50 positive cells in the hippocampus have been reported to have about 30% less mRNA than Alz-50 negative cells, again suggesting that Alz-50 marks dysfunctional neurons (Doebler et al., 1988).

It is unknown whether amyloid deposition precedes, accompanies, follows, or is correlated to tangle formation in Alzheimer disease. Data from Dr. Mann's laboratory suggests that amyloid deposition precedes tangles (Mann and Esiri 1989), but other groups have reported the opposite (Bigio, et al., 1990). Our own data suggest that this is dependent on where you look: in the hippocampal formation and entorhinal cortex, neurofibrillary tangles may precede senile plaques, whereas the opposite is probably true in most cortical areas.

Molecular biological studies of Down syndrome and Alzheimer disease

Why does trisomy 21 lead to early development of Alzheimer disease? The most likely explanation is that the amyloid precursor protein gene resides on chromosome 21 (Robakis et al., 1989; Tanzi et al., 1987a; Goldgaber et al., 1987). Insight into the mechanism of why this is important has come from the genetic study of familial Alzheimer disease. Alzheimer disease is itself inherited in some families, independent of Down syndrome, and although there is clearly heterogeneity the gene

defect in some families lies on chromosome 21 (St. George-Hyslop et al., 1990). Although the great majority of these families do not demonstrate tight genetic linkage with the amyloid pr cursor protein gene (Tanzi et al., 1987b; Tanzi et al., in preparation), a recent exciting breakthrough has been the discovery that some Alzheimer families do have a mutation in the amyloid precursor protein that co-segregates with the disease (Goate, et al., 1991). This suggests that a defect in the amyloid precursor protein alone is sufficient to cause Alzheimer disease.

More direct proof of this idea has emerged in the last year from studies of transgenic mice that have been genetically engineered to "overproduce" amyloid precursor protein (or a fragment thereof). Cordell and colleagues (1991) showed doposition of β/A4 amyloid in animals that had extra copies of the Kunitz-protease containing amyloid precursor protein gene. More dramatic Alzheimer-like pathology, including neurofibrillary tangle like structures, was reported by Kawabata, Higgins and Gordon (1991) in animals transgenic for a fragment of the amyloid precursor protein. Neurofibrillary tangle-like structures were also reported in rats whose brains has been injected with β/A4 amyloid protein itself (Kowall et al., 1991).

Concluding remarks

These data suggest that individuals with Down syndrome will develop neurofibrillary tangles, and senile plaques in their 40's, and that th continued accumulation results in cognitive decline by the 50's or 60's. These occur in the same locations, and are the same biochemically and immunohistochemically, as the neurofibrillary tangles and senile plaques that characteriz Alzheimer disease.

Experiments reported within the past year provide several lines of evidence that the crucial

component of the illness that predisposes Down patients to early Alzheimer-type pathology is the implication of the amyloid precursor protein gene on chromosome 21. This leads to accumulation of β amyloid and presumably, in a way not yet understood, to the development of neurofibrillary tangles in vulnerable populations of neurons. This leads to destruction of neural systems, and to ultimately cognitive impairment after compensatory mechanisms fail. Thus, treatment to prevent the development of Alzheimer disease in Down patients (and perhaps in the general population as well) will be aimed at reducing the expression and/or neurotoxic effects of β-amyloid.

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DOWN SYNDROME AS A KEY TO THE TIME SEQUENCE OF CHANGES IN ALZHEIMER DISEASE

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INTRODUCTION

Extracellular deposition of amyloid and intran
accumulation of abnormal filam nts are the neuropatl
hallmark of Alzheimer disease (AD). Amyloid d p
takes place in the neuropil as well as in the w
leptomeningeal and parenchymal vessels,
accumulation of abnormal filaments occurs within pe
and neurites of selected neuronal populations. Amyl
composed of 4-8 nm fibrils, whos ß-pleated sheet mo
conformation is responsible for green bir fringe
polarized light after Congo red staining and
fluorescence following thioflavine S treatment (G
1980). These tinctorial and optical properties also
to the intraneuronal abnormal filaments that
ultrastructurally of paired helical filaments (PH
15 nm straight filaments. The main component of AD
is a 39-42 residue self-aggregating peptid designa
ß-protein, A4 or Aß (Glenner and Wong, 1984a; Mast
al, 1985; Prelli et al, 1988; Husby et al, 1991) whi
fragment of membrane-associat glycoprot ins (
precursor proteins, APP) of 695, 714, 751 or 770
acids, respectively (Kang et al, 1987; Kitaguchi
1988; Ponte et al, 1988; Tanzi et al, 1988; Selkoe
1988; Golde et al, 1989). The biochemical composit
PHF is not completely elucidated du to th ir insolu
however, evidence suggests that abnormally phospho

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Treatment of mild cognitive impairment: rationale, present and future strategies

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Mild cognitive impairment (MCI) is a condition with a high conversion rate to Alzheimer's disease (AD), which justifies early diagnostic and therapeutic interventions. At the moment, treatment strategies for AD could be extrapolated to interventional strategies in MCI. This article reviews currently available symptomatic treatments with acetylcholinesterase inhibitors, putative treatments such as antiglutamatergic drugs, nootropics, antioxidants, anti-inflammatory drugs and still controversial estrogen replacement therapy, and visionary treatments targeting neuropathological substrates of the disease, such as amyloid production and aggregation, phosphorylation of tau, formation of neurofibrillary tangles and apoptosis. Findings from epidemiological studies have expanded our knowledge on risk as well as possible neuroprotective factors and given means to develop preventive strategies with antihyperlipidaemic drugs such as statins. A wide range of suggested treatments and their possible combinations necessitate their efficacy assessment in well-designed randomized clinical trials where the crucial prerequisites are selection of the treatment population and definitions of outcome measures. Prevention and disease-modifying strategies are raising ethical questions because interventions are focused on nondiseased elderly at risk, which means that emphasis should be not only on efficacy but also on long-term safety.

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Key words: mild cognitive impairment; Alzheimer's disease; treatment

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Previous articles in this Supplement have reviewed evidence that mild cognitive impairment (MCI) is a preclinical dementia based on epidemiological, clinical and neuroimaging studies. However, depending on various terms used for defining MCI as discussed in a paper by Palmer et al. (1), a certain proportion of these subjects also remain cognitively stable for a longer period of time or even improve. Is it therefore plausible to discuss the treatment of MCI with these methodological and diagnostic dilemmas in mind? Is the goal of this treatment to improve cognitive functioning or to delay or prevent the development of Alzheimer's disease (AD), or both? What are the therapeutic options for these individuals at the moment? What does the future promise? How can we evaluate the efficacy of these interventions in subjects at risk, but still without manifest disease? Using various definitions of MCI and various instruments to detect MCI will clearly affect the results of clinical trials in MCI.

Should we consider other possible outcomes and not only AD? Alternatively, should universal neuroprotective interventions at this stage overcome this conventional clinicopathological approach to different dementia syndromes?

This article discusses the rationale and strategies of the treatment of MCI. With this aim in mind, we review studies on neuropathological findings in MCI, briefly present currently accepted concept of a pathophysiological cascade leading to cognitive impairment and dementia, and discuss possible targets for interventions, both symptomatic and disease modifying.

We have integrated articles available in PubMed satisfying evidence class I and II, which show major research directions in the field of treatment of dementia, namely AD therefore, these could be extrapolated to interventional strategies in MCI. The current article is not meant to be a detailed review of this field but, rather, a summary and a

point of departure for the broader medical audience for further search and discussions on this topic.

Neuropathology of MCI: rationale for treatment

Several clinicopathological correlative studies support the construct validity of the MCI concept (2–5). They all agreed that individuals scoring 0.5 on the Clinical Dementia Rating (CDR) scale had enough pathology in the brain to satisfy the AD diagnosis, which suggests that, at the time when dementia is not fully clinically expressed, the disease already exists at the histopathological level. In 10 autopsied subjects with 'questionable' dementia, Morris et al. (2) reported the presence of histopathological markers of AD in the neocortex: neurofibrillary tangles (NFT) and high total plaque density with a preponderance of the diffuse plaque subtype. None of the four control subjects in this study, with ages in the range 76–89 years, had enough NFT and neuritic plaques (NP) to establish an AD diagnosis. A number of studies investigated neuronal numbers in vulnerable structures such as the hippocampus and entorhinal cortex, and they showed severe neuron loss in the brain of MCI subjects (CDR 0.5) in layers II and IV of the entorhinal cortex in particular (3, 4). The study of Price et al. (5) also focused on cell loss in the brains of 13 healthy nondemented persons: four preclinical AD cases rated as CDR 0, and eight very mild AD cases rated as CDR 0/0.5 or 0.5. Although preclinical AD cases in this study had substantial numbers of plaques and tangles, they did not differ from healthy nondemented cases in the volume and number of neurons in the entorhinal cortex and the hippocampal CA1 field. These findings indicate that the pathophysiological process leading to AD is active in the very early preclinical stage, but still does not cause significant neuronal degeneration and death. In the future, if biological markers or instrumental investigations specific and sensitive for this very early stage of the disease become available, then the disease-modifying interventions targeting pathological substrates could be applied in this ideal therapeutic window that precedes irreparable neuronal loss and death.

Pathophysiological cascade leading to cognitive impairment

Our current understanding of the pathophysiological processes leading to AD is a complex cascade of

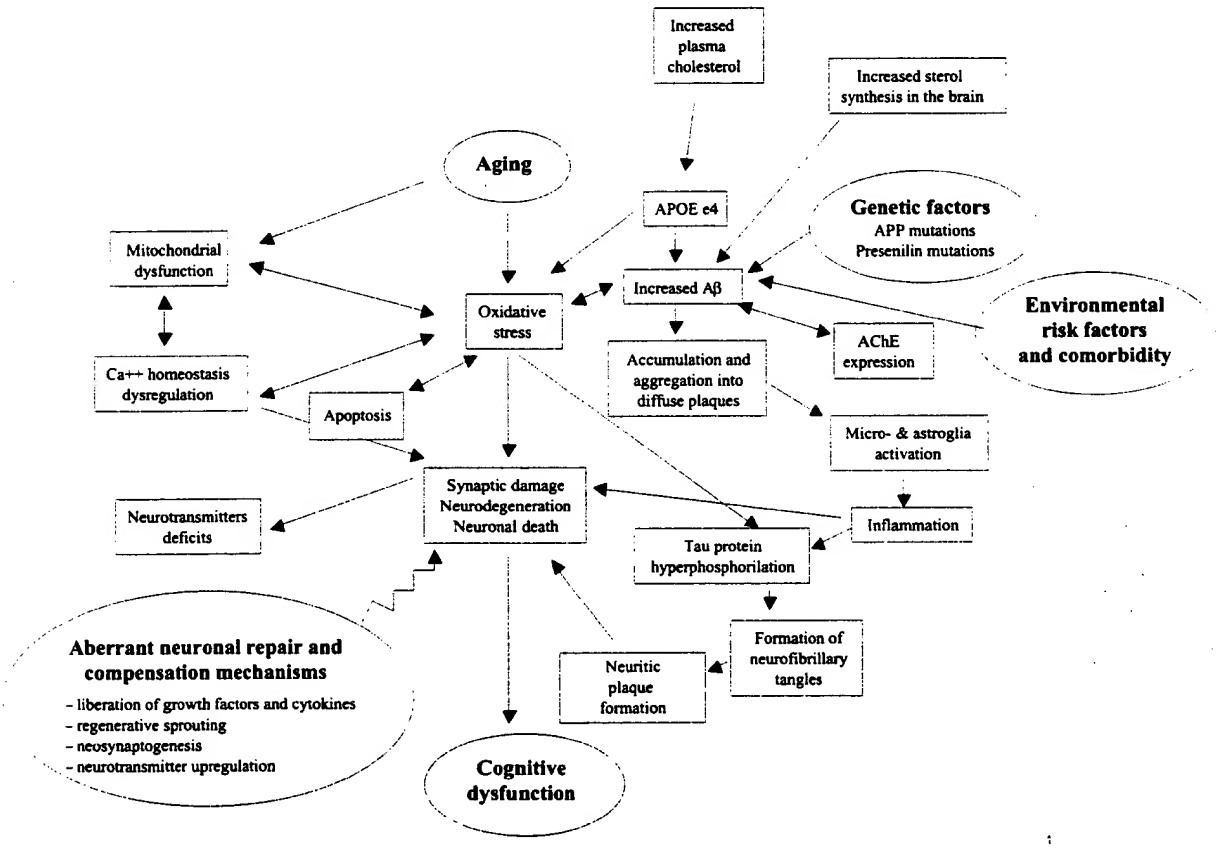

Figure 1. Pathogenesis of Alzheimer's disease: cascade of molecular events and complex network of interactions among aging-related processes, genetic and environmental risk factors and neuronal repair and compensation mechanisms.

events at the molecular level (Fig. 1). Aging, which is the major risk factor for dementia, sets the stage for the compromised homeostasis of calcium, mitochondrial energy, and free radical production (6). These aging-related processes in combination with major genetic risk factors (namely amyloid precursor protein (APP) and presenilin mutations) lead to synaptic damage, neurodegeneration and neuronal death through oxidative stress, the consequences of which are neurotransmitter deficits, microglial activation and inflammatory responses. Two major pathological lesions characterizing AD are products of this chain of events: NP and NFT. The neuritic plaques are extracellular deposits comprising a core of aggregated β-amyloid (Aβ) surrounded by degenerating neurites, activated microglia and reactive astrocytes, while NFT are intraneural deposits of hyperphosphorylated microtubular protein tau.

At present, available treatments are operative at the very end of this cascade of events, focusing primarily on neurotransmitter substitution (Fig. 1). Future therapies will probably act on the level of more proximal events and target biological substrates of the disease, NFT and NP (7).

Currently available treatments

Acetylcholinesterase inhibitors

The acetylcholinesterase (AChE) inhibitors are presently the established treatment strategy in Alzheimer's disease and are considered as first choice candidates for the treatment of MCI (Table 1). This intervention is based on the cholinergic hypothesis of AD (8), which is built on the findings of cholinergic deafferentation of the cerebral cortex as a result of the selective loss of cholinergic neurons in the basal forebrain (9, 10) as well as on the positive effects of double-blind placebo-controlled trials of up to 26 weeks duration with five AchE inhibitors (11). The most recent autopsy-based study reported similar reductions in basal forebrain immunoreactive neurons in individuals with MCI and AD (12). Three acetylcholinesterase inhibitors (donepezil, rivastigmine and

Table 1 Treatment strategies in AD: presently available, putative treatments and future interventions in MCI

	Mode of action		
Type of intervention	Symptomatic	Disease modifying	Prevention
Currently available			
Cholinergic drugs	+	–	–
AChE inhibitors (donepezil, rivastigmine, galantamine)			
Putative treatments			
Antiglutamatergic drugs	+	–	–
NMDA-antagonists (memantine)			
Antioxidants	–	+	–
E-vitamin			
Ginko biloba			
MAO inhibitor (selegiline)			
Nootropics, piracetam	+	–	–
Anti-inflammatory drugs	–	–	+
NSAID			
COX-2 inhibitors (celecoxib)			
Hormonal substitution			
Estrogen replacement therapy	–	–	?
Novel approaches under development			
NGF & neotrophines	–	+	–
Idebenone			
ATT-082			
Interventions in β-amyloid processing	–	+	–
Aβ-vaccination (with a nontoxic/nonfibrillar amyloid-β)			
γ secretase inhibitors			
α secretase enhancers			
β secretase inhibitors (BACE 1)			
Interventions in tau-hyperphosphorylation			
Glycogen synthase kinase 3β inhibitors	–	+	–
Comorbidity treatment, risk factors control			
Antihypertensive therapy	–	–	+
Statins	–	–	+
lovastatin, pravastatin			
Vitamin B12 & folate supplementation	–	–	?

galantamine) are approved and available on the market in many countries, while tacrine has become obsolete because of its unacceptable side effects. Although these drugs differ in their selectivity for AChE and in their pharmacokinetics, they appear to be similar in their clinical effects (13). Their efficacy has been evaluated separately across three key domains of AD – cognition, behaviour and activities of daily living (ADL) – and short symptomatic improvements up to 1 year have been reported (14). Possibilities of long-term effects via modification of APP metabolism have also been discussed (11) and studies designed to measure long-term efficacy of AchE inhibitors are ongoing. Large multicentre trials are currently in progress, with the aim of evaluating whether donepezil (conducted by the National Institute on Aging) and rivastigmine (Investigation into Delay to Diagnosis of Alzheimer's disease with Exelon, InDDEx) have an effect on the conversion rate from MCI to AD (15). MCI subjects in the InDDEx study are selected according to criteria that include CDR scoring 0.5, delayed recall test and exclusion of depression as evaluated by the Hamilton depression scale.

However, recent studies have challenged the prospects of AChE inhibitors as a treatment option for MCI. Davis et al. (16) reported that cholinergic deficits became apparent later during the course of AD, and DeKosky et al. (17) observed regionally specific uppregulation of choline acetyltransferase in MCI subjects. The latter study suggests compensatory processes in the preclinical phase of the disease and suggests limitations of AchE inhibitors efficacy at this stage of the disease.

Putative treatments

Antiglutamatergic drugs

One of the causes of neurotoxicity is overactivity of the excitatory amino acid glutamate (18). By blocking its binding site at N-methyl-D-aspartate (NMDA) receptor sites, excitotoxic damage could be prevented or ameliorated. NMDA-mediated excitotoxicity has been shown to increase tau phosphorylation and, therefore, has been related to the formation of NT – one of the major pathological substrates of AD (19). Memantine, an NMDA-receptor antagonist, has been in clinical use in Germany for nearly two decades and was one of the first drugs applied in AD. Clinical trials with memantine have so far been designed to show symptomatic effects and two studies have reported its efficacy in advanced AD (20, 21). Preclinical data are pointing in favour of possible neuroprotective and, therefore, disease-modifying effects; this means that high-risk populations such as MCI subjects could benefit from this treatment in the future (22, 23).

Nootropics

Setting memory enhancement as a therapeutic goal has give rise to new interest in nootropic agents and piracetam as the most commonly used representatives of this class of drugs. Mainly symptomatic effects could be expected, because possible modes of action are nonspecific and include effects on energy metabolism, cholinergic mechanisms, excitatory amino acid receptor-mediated functions and steroid sensitivity (24). Piracetam was used in a clinical multicentre, multiphasic, cross-over double-blind trial with nondemented patients with memory impairment, and positive effects were reported on tests of attention and memory (25). Inclusion criteria in this study were more lenient than the current concept of MCI: patients were included only if they had a Hachinski Dementia Score less than 33, Mini-Mental State Examination (MMSE) score less than 30, Hamilton Rating Scale for Depression score less than 18, Token Test score more than 28, difficulties in concentrating and in the execution of habitual daily living occupations or changes in the personality or behaviour. A combination of piracetam and memory training in patients with age-associated memory impairment (AAMI) was also effective in a double blind, randomized trial (26). The most recent meta-analysis of the clinical efficacy of piracetam in cognitive impairment included 19 double blind, placebo controlled studies with clinical global impression of change as a common outcome measure (27). The results of this study demonstrated improvement in older subjects with diverse cognitive impairment treated with piracetam as compared with placebo. To justify a revival of piracetam for possible treatment of MCI, more trials are needed with more adequate selection of the study group and definition of outcome measures.

Antioxidants

Oxidative stress has been proposed as a central pathogenetic mechanism in various neurodegenerative diseases, including AD (28–31). Large amounts of unsaturated lipids and catecholamines in the brain are particularly vulnerable to free radical damage. β-protein precursor, Aβ, presenilins and apolipoprotein E are linked to reactive oxygen species (ROS) production and apoptosis. In addition, oxidative stress is also recognized as a contributing mechanism in atherogenesis (32), which increases the risk of cognitive impairment through the process of atherosclerosis and thrombosis.

A longitudinal population-based study of older subjects showed that high plasma levels of lipoperoxidation markers were a significant risk factor for cognitive decline during the 4-year follow-up period (33). Another study, performed among people aged ≥65 years, found that higher ascorbic acid and β-carotene plasma levels were associated with better memory performance (34). These results suggest an important role of antioxidants in brain aging and possible prevention of cognitive impairment with dietary measures. However, there are concerns that dietary intake does not guarantee central availability of the antioxidants. Currently available therapies directed towards the reduction of oxidative stress include the elimination of free radicals through interaction with free radical scavengers, and prevention or decrease of their production by antioxidants (35). Ginko biloba, and vitamins A, C and E act as free radical scavengers, while selegiline, an MAO inhibitor, reduces free radical formation and, thus, represents antioxidants.

In experimental studies, vitamin E has protected hippocampal cells from degeneration following cerebral ischaemia (36). In a recent *in vitro* study, lipoperoxides suppressed the nicotinic receptors and the suppression induced by Aβ could be prevented in the presence of vitamin E (37). In a clinical study where Alzheimer patients received vitamin E (2000 IU per day) for 2 years, there was a delay until institutionalization or death in the patients compared with the placebo group (38). A similar effect on the progression of the disease was observed in a group of Alzheimer patients treated with the monoamine oxidase inhibitor selegiline (35). This study included four primary end-ponts: death, institutionalization, loss of basic ADL and diagnosis of severe dementia and secondary outcome measures of cognition, function, behaviour, and the presence or absence of extrapyramidal signs. Cognition was assessed with the cognitive portion of the Alzheimer's Disease Assessment Scale (ADAS-cog) and the MMSE. Opposite to findings with cholinesterase inhibitors (39), no improvement in cognitive functioning was observed in patients receiving long-term treatment with vitamin E or selegiline (38). However, patients in this study were in a moderately severe stage of the disease, probably already with extensive neurodegeneration and neuronal cell death. Future intervention trials should evaluate disease-modifying properties of these compounds by the inclusion of subjects at risk of developing dementia, i.e. those with MCI. In addition, methods studying antioxidant exposure through dietary intake should be developed and applied on larger epidemiological cohorts followed for a longer periods of time (40).

Recently, two independent observational studies found an association between dietary intake of vitamins E and C and reduced risk of developing AD (41, 42). It seems plausible that neuroprotective properties of antioxidants are better expressed through the long-term exposure via dietary intake, which also could have protective effects on other comorbid processes such as atherosclerosis, than during the short-term clinical trials with high doses of supplements.

Although there is no consensus in the scientific community and regulatory bodies on therapeutical efficacy of Ginko biloba, it is one of the most used antidementia substances, which, for example, in Germany holds more than 50% of the market (43). Low cost and widely spread belief that phytopharmaca are free of side effects are major causes for self-medication, which happens on a large scale. Therefore, there is a need for more well-designed clinical trials with proper choice of outcomes (44). Results of a randomized, double blind, placebo controlled multicentre trial with the Ginko biloba extract have been reported recently (45). They indicate a favourable treatment effect with respect to cognitive performance and social functioning. Relative changes from the baseline measured after a 52-week trial duration show improvement in patients with mild or very mild cognitive impairment, while effects in those with more severe dementia were understood as stabilization or slowing down the progression.

Anti-inflammatory drugs

There is increasing evidence that inflammatory processes are involved in the pathogenesis of AD (46), although there is still some debate as to whether they are the primary process causing the brain damage or just reaction after another more proximal process in the pathogenetic cascade. Experimental studies suggest that there is an upregulation of inflammatory cytokines and acute phase proteins, activation of the complement regulatory proteins as well as an accumulation of activated microglia (46), which is also revealed at autopsy in brains of AD patients in association with amyloid plaques. Epidemiological studies have reported a reduced prevalence of AD in patients with arthritis (47). The finding suggests that exposure to anti-inflammatory drugs should, to some extent, protect against AD (48). A reduced risk of AD has also been observed among users of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) (49–51). Clinical studies with indometacin showed some signs for arrest in decline of mental functioning of treated Alzheimer

patients (52). Treatment with COX-2 inhibitors might be promising, because it has been suggested that COX-2, which is expressed in the brain, can be involved in response to neurodegenerative stress (53). An early initiation of treatment before patients develop clinical AD might be crucial for the outcome of treatment studies with anti-inflammatory drugs. The Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT) with celecoxib, selective COX-2 inhibitor and naproxen is ongoing. This study was designed as a primary prevention trial in subjects that are at increased risk for AD due to their advanced age (> 70 years) or family history (54).

Estrogen replacement therapy (ERT)

Some epidemiological studies have reported a reduced incidence of AD in postmenopausal women on ERT (55). However, data from various observational studies and clinical trials are still inconclusive, possibly because of methodological differences (56). Possible beneficial effects of estrogen on cognition have been suggested in several studies and specific protective effects have been observed in the verbal memory domain (57, 58). Via its receptors ERα and Erß, estrogen has been found to cause multiple effects in the brain, including the activation of nerve growth factors (NGFs), increased synaptogenesis, modulation of function of several neurotransmitters, including acetylcholine, serotonin, dopamine, noradrenaline as well as increase of cerebral blood flow (59, 60). Because of an intrinsic antioxidant activity, as well as a neuroprotective effect obtained by promoting the nonamyloidgenic β-secretase processing of APP, estrogen may play an important role in nerve cell survival (60). Therefore, estrogen-like substances might be of interest to develop for the treatment of AD. The use of estrogen was recently shown to be associated with less cognitive decline in women carrying apolipoprotein E (APOE) e 4 compared with noncarriers of e 4 (61). In the first large clinical trial with estrogen administered to female Alzheimer patients for 1 year, ERT showed no positive effects (62). A recent large population-based study, including over 100 000 individuals, added to this controversy by showing that postmenopausal ERT was not associated with a reduced risk of AD (63). However, to determine whether ERT has clinically significant protective effects and whether benefits outweigh the risk, more placebo-controlled studies with large sample sizes and well-controlled confounding factors are needed. These (preferably multicentre) studies should examine effects of different dosages, routes of administration, length of treatment, as well as possible synergistic effects of combination with other preventive therapies. Furthermore, effects of newer, more specific selective estrogen modulators should be explored and brain imaging should be included as a more sensitive outcome measure to evaluate treatment effects.

Visionary interventions

The ultimate goal of early therapeutical interventions in individuals at risk of developing dementia is to prevent, delay or slow the progression of the disease (7). These disease-modifying effects could be expected only if more proximal and central events in the pathogenetic cascade and their substrates are ameliorated, such as amyloid production and aggregation, phosphorylation of tau, formation of NFT, and apoptosis (Table 1).

Targeting neuropathological substrates

According to the amyloid cascade hypothesis, reduction of Aβ production could be achieved by the inhibition of two key enzymes, which play a role in the cleavage of toxic Aβ fragment and its deposition in the form of plaques. Difluoroketone inhibitors of gamma-secretase have now been synthesized and are approaching clinical testing (64). An APP-specific β-secretase (BACE for beta-site APP-cleaving enzyme) might be another promising target for the development of Alzheimer drugs (65). Inhibitors of Aβ aggregation, such as beta sheet breakers, will probably reach the clinic within a few years. However, the amyloid cascade hypothesis has been challenged recently, because APP transgenic animals develop behavioural abnormalities before extensive amyloid deposition occurs (66). These findings are also in line with earlier studies that reported that the extent and distribution of neurofibrillary changes better correlated with disease severity than amyloidosis (67, 68).

Immunization therapy has offered an opportunity to test the validity of the amyloid cascade hypothesis and whether accumulation of amyloid is critical for cognitive deficits in AD. It is based on findings of Schenk et al. (69) that, after immunization with the Ab42 fragment of APP, cerebral amyloidosis was largely prevented in transgenic mice with an overexpression of a mutant form of human APP. The first clinical trial involving the vaccination of AD patients had to be stopped in Phase II, because of observed unacceptable side effects related to brain inflammation and oedema in some patients. Although the trial has been suspended, the concept has not yet been abandoned. There are already reports that immunization with a nontoxic, nonfibrillar Aβ

derivative reduces AD pathology in transgenic mice without causing inflammation (70).

Most recently, the discovery of a new small-molecule drug that interferes with the serum amyloid P component (SAP) has been announced (71). SAP binds to amyloid deposits, promotes their aggregation, stabilizes them and protects from degradation. If this drug proves in the future to inhibit or reverse amyloid fibilogenesis, having the additional advantage of being nontoxic and well tolerated, it will give hope not only to the patients with system amyloidosis but also to those at risk of developing AD.

Although our current understanding of the pathophysoiology of AD puts amyloid deposition before tau in the cascade of neuronal damage, intervention in tau hyperphosphorylation should not be disregarded. Possibilities to block hyperphosphorylation of tau are through inhibition of glycogen synthase 3-β (GSK 3-β) and cyclin-dependent kinase 5 (CDK-5) (Table 1).

Regulation of neuronal plasticity

Neuroplasticity failure has been proposed as a theory to unify the link between amyloid aggregation and NFT formation (72), which raises the consequent dilemma of which of these pathological substrates to target with future therapies. It is hypothesized that multiple factors increase the neuroplasticity burden, which initially causes adaptive upregulation of tau phosphorylation and APP turnover, and triggers plaque and NFT formation. For this reason, interventions in β-amyloid processing and tau-hyperphosphorilation might not be successful according to our expectations.

NGF and neurotrophines are alternative neuroprotective approaches still under development, which promise to regulate neuronal plasticity by controlling neurotransmission, connectivity, and neuritic outgrowth. The hypothesis behind the intervention with NGF is based on findings that NGF acts as a strong neurotrophic factor for the basal forebrain cholinergic neurons (73) and ameliorated learning and memory impairment in rats with basal forebrain lesions. There are already some empirical data on NGF application in humans. Intraventricular administration of NGF to three Alzheimer patients for 3 months showed an increase in nicotinic receptor binding measured by PET, while a clear-cut cognitive amelioration could not be shown (74). The dose infused intraventricularly to Alzheimer patients was similar to the NGF dose earlier infused intraputaminally to patients with Parkinson's disease (75). However, in Alzheimer patients, it caused pain and weight loss (74). Histopathological studies have described dystrophic neuronal growth in AD brains, which is most probably a compensatory mechanism of neuronal reorganization that leads to the aberrant neuronal repair (76). Therefore, a strategy of system application of neurotrophic factors seems to have a conflict within a concept *per se* and requires more knowledge about processes that induce and control neuronal repair. This implies that alternative administration routes should be explored, other than invasive intraventricular infusion with unacceptable side-effects. Gene therapy enables NGF delivery in a highly specific spatial fashion to the most vulnerable brain regions, i.e. transplantation of fibroblasts modified to secrete NGF into the basal forebrain (77), a treatment already applied a year ago in an AD patient.

These invasive methods are still far from clinical application and they will be reserved for the individuals at highest risk of developing the disease. Neotrophines are orally active NGF stimulators and therefore more preferable. Their efficacy should be further explored in combination with other available noninvasive pharmacotherapies mentioned in this article.

Treating comorbidity, controlling risk factors

Clinical expression of dementia is supposedly determined, on the one hand, by a balance among genetically determined disease processes, aging-related cognitive decline, environmental risk factors and comorbidity and, on the other hand, by neuronal repair and compensation mechanisms (Fig. 1).

Identification of risk factors and expanded knowledge on their interactions may, in the future, explain the heterogeneity of the MCI population in terms of its clinical presentation and natural history. This will also initiate thinking about new therapeutical approaches and various neuroprotective strategies.

The presence of vascular risk factors during midlife in a large longitudinal population-based study was related to late-life MCI (78). This study showed that individuals with raised systolic blood pressure or high serum cholesterol concentrations had significantly higher risk of developing AD in later life (79). Another study reported a significantly increased risk of MCI in subjects with elevated midlife diastolic blood pressure, white matter hyperintensities (WMH) and the presence of apolipoprotein E4 genotype (80). Because vascular disease is an important contributor to dementia development, identification and control of treatable vascular risk factors in asymptomatic subjects as well as in those with MCI could delay clinical expression of dementia. It has been suggested in the large

community-based population of the Kungsholmen project that antihypertensive medication with diuretics protects against dementia in elderly persons by a 40% reduction in relative risk (81). Another study from the same cohort found a significant association between incident AD and low serum levels of vitamin B12 and folate (82). A report that increased serum homocysteine – a marker of vitamin B12 and folate deficiency – is an independent predictor of cognitive decline in healthy elderly subjects further supports these findings (83). It has been proposed that oxidative stress impairs the metabolism of homocysteine and that, under these conditions, only particular forms of vitamin B12 supplements, such as glutathionylcobalamin, could be beneficial as a prevention strategy (84).

Recently, three retrospective epidemiological studies have raised a lot of interest by showing that statins, i.e. cholesterol-lowering agents, decreased the risk of developing AD by 70% (85–87). Furthermore, an observational study of 1037 postmenopausal women with coronary heart disease reported that high levels of low-density lipoprotein (LDL) and total cholesterol were associated with cognitive impairment (88). A population-based autopsy series of 218 Japanese American men found a strong dose–response association of late-life high-density lipoprotein (HDL) cholesterol with the number of NP and NFT (89). Increased plasma cholesterol may be associated with an increase in the rate of Aβ production, an increase in the level of APOE and, consequently, an increase in NP formation (90). Comparison of statin users with nonusers showed that the users had a higher mean score on the modified MMSE (87). Interestingly, these findings seemed to be independent of lipid values. The protective mechanism of statins probably acts beyond the lowering of lipids and possibly is mediated via effects on microvasculature and cerebral blood flow.

Interventions beyond pharmacotherapy

Scientific topics and latest findings are becoming available to the general public through new information media, often causing increased anxiety to individuals about their current and future health status. Consequently, healthcare systems will have to cope with well-informed and 'well-worried' older people searching for reassurance or optimal interventions regarding their memory loss. Do we have evidence-based recommendations for a life style that could be a primary prevention for dementia? Epidemiological studies have shown that extensive social network and participation in cognitively stimulating activities are associated with a reduced risk of AD (91–93). The frequency of cognitive activity was not only associated with the baseline level of cognition, but also with the rate of cognitive decline (93). It is therefore possible that emotional and mental stimulation in elderly people could delay the clinical manifestation of the disease. Could it be possible to design cognitive training for individuals at risk? Very few studies have addressed this problem, which lies in the borderline zone between cognitive science and neuropsychology of aging. The most recent study designed as a randomized clinical trial evaluated effects of multifaceted memory enhancement and relaxation training for older adults with MCI (94).

At the 6-month follow-up, trained individuals had significantly better memory appraisals and showed a trend toward better word list recall than did controls. There is a need to further develop these programmes and test their efficacy in MCI individuals alone or in combination with other available treatments. Furthermore, establishing the range and limits of this cognitive reserve capacity in the elderly by means of such training could become a new diagnostic strategy for the early identification of AD (95).

Evaluation of treatment efficacy in MCI

A wide range of suggested treatments and their possible combinations necessitate their efficacy assessment in well-designed randomized clinical trials. The crucial prerequisites are the selection of the treatment population and the definitions of outcome measures. Selection criteria should be enriched with knowledge concerning the power of various neuropsychological, genetic and laboratory tests in predicting conversion to dementia, i.e. AD. Outcome measures should cover key domains of disease phenomenology, be sensitive to detect clinically meaningful change in early stages of the disease, and be user friendly in clinical and drug-trial settings (Table 2).

Recently, a modified version of the cognitive subscale of the ADAS-Cog, which includes tests of delayed recall and executive functioning, has been developed to increase the usefulness of this scale in short-term trials with MCI patients (96). Because there is a possibility of learning effects, there should be alternative cognitive measures developed for repeated testing. Clinical rating scales, CDR and GDS, which function as global severity measures, have been criticized for their lack of sensitivity for the very early and late stages of the disease. However, CDR suffers less from this criticism and still remains in use among other selection criteria in drug trials. Secondary outcome measures should

Table 2 Outcome measures related to key symptom domains and neuropathological features in AD*

Aspect	Outcome measure
Cognition	ADAS-Cog (modified)
	MMSE
ADL	ADCS/ADL
	DAD
	PDS
Behaviour	NPI
	BEHAVE-AD
Global function	CIBIC-plus
	MCI-CGIC
	CDR
	GBS
Atrophy	MTL volumetry
CSF markers	Tau protein
	β-amyloid
	Oxidation products?
	Inflammatory markers?

*Modified from (44).

ADAS-Cog, Alzheimer's Disease Assessment Scale-cognitive subscale; MMSE, Mini Mental State Exam; ADCS/ADL, Alzheimer's Disease Co-operative Study/Activities of Daily Living; DAD, Disability Assessment for Dementia; PDS, Progressive Deterioration Scale; NPI, Neuropsychiatric Inventory; BEHAVE-AD, Behavioral Pathology in Alzheimer's Disease Rating Scale; CIBIC-plus, Clinician's Interview-Based Impression of Change – plus caregiver input; MCI-CGIC, Mild Cognitive Impairment – Clinician's Interview-Based Impression of Change; CDR, Clinical Dementia Rating; GBS, Gottfries-Bråne-Steen scale; MTL, medial temporal lobe.

include change in biological markers of the disease, i.e. biochemical or neuroimaging, reflecting modification in a key pathological feature of the disease. Since disease modification is the preferential treatment goal in MCI, survival design is the optimal trial design because, as a result, it gives time to reach a clinical milestone like conversion from MCI to dementia. Placebo controls are becoming obsolete due to ethical reasons, and active-control designs in AD should be used to demonstrate eventual superiority of a new drug to standard drugs (96).

Summarizing remarks

MCI is a condition with high conversion rate to dementia, i.e. AD, which justifies early diagnostic and therapeutic interventions. Symptomatic treatment will have limited success in early stages of the disease, because it cannot reverse the already existing disease process and neuropathological changes in the brain. Disease-modifying strategies are derived from two sources: knowledge about molecular pathogenesis of the disease and epidemiology. New insights into the molecular pathogenesis recognize amyloid, tau and oxidative stress as the main targets for the intervention, while findings from epidemiological studies expand our knowledge on risk as well as possible neuroprotective factors. Current therapeutic strategies for MCI are based on those for AD, although MCI can have differential outcomes.

Prevention and disease modifying strategies are raising ethical questions because interventions are focused on nondiseased elderly at risk. Therefore, emphasis should be not only on efficacy but also on long-term safety.

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